THE DISTRIBUTION OF YELLOW FEVER IMMUNITY IN NORTH AMERICA, CENTRAL AMERICA, THE WEST INDIES, EUROPE, ASIA, AND AUSTRALIA, WITH SPECIAL REFERENCE TO THE SPECIFICITY OF THE PROTECTION TEST

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A survey of the world for yellow fever immunity in man has been carried on during the past five years by the International Health Division of The Rockefeller Foundation with the co-operation of the many governments concerned. The surveys of Africa and South America produced evidence of a much wider distribution of yellow fever than had been suspected, and required separate reports. The final report for Africa has been published by Sawyer and Whitman (1) with a review of the results of the earlier participation in the survey by Beeuwkes and his associates (2, 3) and Stefanopoulo (as reported by Boyé (4)). The survey of South America by Soper and his colleagues is not yet complete, but preliminary reports may be found in publications of Kerr and Patiño Camargo (5), Sawyer (6), and Soper (7). In the other parts of the world investigations were made in as many countries as seemed necessary to determine the general boundaries between the recently infected and the non-infected regions and to find out where intensive local investigations were required. The communication now being presented is the final report on these surveys outside of Africa and South America and includes observations for North America, Central America, the West Indies, Europe, Asia, and Australia. Reports have been made for some of the countries by Hughes and Sawyer (8), and Sawyer (6), but the essential findings of the earlier investigations have been combined with those now set forth.

The immunity survey was possible because an attack of yellow fever leaves a lasting immunity, the presence of which can easily be demonstrated. To determine the presence of immunity, a specimen of the blood serum of the person to be tested is injected together with a relatively large amount of living virulent yellow fever virus into a susceptible animal. The survival of the test animal is taken as an indication that specific immune substances, the result of a previous attack of yellow fever, were present in the serum and protected the animal against a fatal infection.

Shortly after the discovery of an animal susceptible to yellow fever by Stokes, Bauer and Hudson (9), rhesus monkeys were used in such protection tests by Beeuwkes, Bauer and Mahaffy (10) to determine whether yellow fever had been present in certain communities. Because of the excessive cost of monkeys, the extent to which such tests could be applied for epidemiological investigation was necessarily limited. The important discovery by Theiler (11) that white mice are susceptible to yellow fever when inoculated intracerebrally made possible a wider application of the protection test to epidemiological investigations.

The technique used throughout the immunity survey in determining the presence of yellow fever immune substances in serum was that of the intraperitoneal protection test in mice, as worked out by Sawyer and Lloyd (12). The blood specimens were random samples from persons who had lived all their lives in the locality under investigation. As in the African surveys an attempt was made to obtain 25 specimens or more from adults and the same number from children in each locality, but in certain regions in which it was highly improbable that yellow fever had been present in recent years adults alone were tested. If less than 10 sera were tested in one of these age groups in a locality, the results for that age group were omitted from the maps presented with this report, as though no specimens had been collected. In the previous surveys of Africa persons aged 16 years or less were classed in the tables and on the maps as children and those aged 17 years or over as adults, but on the maps in this report persons who were under 15 years of age were considered as children and those aged 15 years or over as adults.

The specimens were collected for us through the kind coöperation of local medical authorities.

SPECIFICITY OF THE YELLOW FEVER PROTECTION TEST AS SHOWN BY THE RESULTS OBTAINED WITH SERA FROM NON-YELLOW FEVER COUNTRIES

From time to time questions have been raised as to the degree of specificity of the yellow fever protection test and the reliability of the survival of mice inoculated with living virus and an unknown serum as an indication of the presence in the serum of yellow fever immune substances acquired as the result of experience with yellow fever virus. Inasmuch as the protection test was being employed extensively in epidemiological investigations, it was considered of prime importance that information be secured regarding its specificity. There was already much evidence that protective antibodies appeared regularly after an attack of yellow fever, but not enough evidence to determine to what extent protective substances might be encountered in the sera of persons who have never been infected with yellow fever virus. With a view to obtaining the needed information and establishing a standard with which the results of the epidemiological surveys could be compared, a large number of serum specimens were tested from widely separated regions in which exposure to yellow fever virus seemed impossible as the disease had never been known to be present. The countries from which these specimens were collected were Australia, Ceylon, China, Java, India, Federated Malay States, Philippine Islands, and Syria. The results are given in table 1 and map I. As seen from this table, only two specimens, or 0.23 per cent, of a total of 876 specimens gave protection against yellow fever virus. Both of these came from India, and as far as we have been able to ascertain neither of the donors had ever been exposed to yellow fever infection.

Although the results indicate that the protection test is highly specific, and the proportion of normal sera showing yellow fever virucidal properties is exceedingly small, the fact that such sera are encountered at all seems to justify a more detailed discussion.

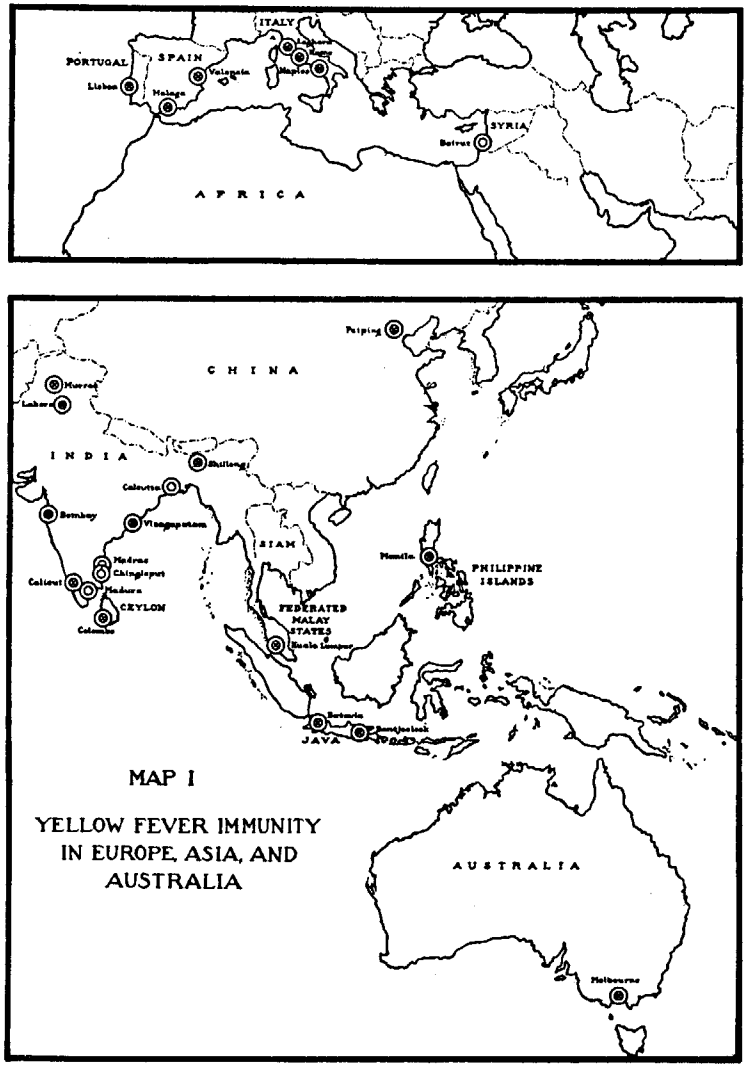
Clear cut and highly specific results are obtained in the intraperitoneal yellow fever protection tests in mice only when massive doses of virus are used. In the early stages of the yellow fever protection test work, a 10 per cent emulsion of infected mouse brain was used as a source of virus. The results were sometimes

TABLE 1

Results of protection test survey in non-yellow fever countries

COUNTRY AND TOWN	AGE GROUPS OF DONORS												TOTAL FOR ALL AGE GROUPS		
	5 to 9 years			10 to 14 years			15 to 19 years			Adults, 20 years and over					
	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive
Australia, Melbourne							5			29			34		
Ceylon, Colombo										44			44		
China, Peiping										118			118		
Java:															
Batavia	2						3			20			25		
Bentjoeloek							1			23			24		
India:															
Madras	2			17			5			64			88		
Chingleput				10			16			61	2	3.3	87	2	2.3
Calicut							2			23			25		
Vizagapatam	1			1			1			58			61		
Lahore							17			17			34		
Murree							2			28			30		
Shillong	2			4			4			9			19		
Calcutta	5			19			6			22			52		
Bombay				9			6			26			41		
Madura				17			15			23			55		
Malaya, Kuala Lumpur							6			46			52		
Philippines, Manila										27			27		
Syria, Beirut	8			7			3			42			60		
Total	20			84			92			680	2	0.29	876	2	0.23

confusing and not clear cut in that a relatively large proportion of specimens gave inconclusive results in which three or four mice of a group of six used for testing a specimen survived. After the amount of the virus used was doubled in September, 1932, and a 20 per cent infected mouse-brain suspension was used, the pro-



MAP I. DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN EUROPE, ASIA, AND AUSTRALIA

Outer circles represent adults. Inner circles represent children. Black sectors show the proportion with protective serum. Circles crossed by diagonal lines signify that no tests, or less than 10, were made in the age group.

portion of sera giving such partial protection became very small and the test became more clear cut and highly specific. The quality of the test was further improved by using only strains of mice which were known to be very susceptible to yellow fever.

Among the large number of specimens received from India, there was one from a Tamil, male, 20 years of age, who lived in Chingleput and had been all his life in the Madras Presidency. This specimen gave an inconclusive result in one test with 10 per cent virus and protection in another. Unfortunately, there was not enough serum left for further studies and, as we have not been able to secure another specimen from this donor, it is listed among the positives in table 1. A specimen giving similar results was obtained from a 13-year-old child of Calcutta. It was tested three times with a 10 per cent virus emulsion, and gave inconclusive results twice and full protection once. A second specimen from the same child secured eight months later was tested against 20 per cent virus and the result was a clear cut negative. The result is therefore classified as negative. Among the group tested from Chingleput, Madras Presidency, there were two specimens which gave full protection on repeated tests with 10 per cent emulsion, both when the serum was undiluted and when diluted 1:10. Both donors were male Tamils who had lived in the Chingleput District all their lives. One of them, "P. B." was 17 years old, and the other, "N," was 40. Second specimens were obtained from both fifteen months after the first bleeding. The serum of "P. B." was tested twice against 20 per cent virus-brain suspension and gave an inconclusive result in one test and a clear cut negative in the other. This specimen is classed as negative in table 1. As there was a time interval of fifteen months between the withdrawal of the first and second specimens and a difference in technique in testing them, it is impossible to determine in retrospect whether the protective substances so prominantly present in the first specimen had diminished in concentration in the course of fifteen months, or whether the negative result with the second specimen was entirely due to the larger amount of virus used in the tests.

The serum of the other positive donor, "N," is of great interest.

As far as we have been able to ascertain, this donor had never been out of India and therefore had never been exposed to yellow fever infection. The first specimen was received in May, 1932, and was tested against 10 per cent virus-brain suspension. It showed full protection undiluted, as well as in dilutions of 1:2 and 1:10. A second specimen which was received in June, 1933, was tested against 20 per cent virus suspension and it protected fully when tested undiluted, as well as in a 1:10 dilution. A dilution of 1:16 gave inconclusive results, and dilutions of 1:32 and 1:64 showed no protection. A third specimen was received in April, 1935, and was again tested against 20 per cent virus-brain suspension. It protected fully when tested undiluted and in 1:2 dilution, while dilutions of from 1:4 to 1:32 gave entirely negative results. Here then is an instance in which the serum of a person who had presumably never been exposed to yellow fever virus exhibited yellow fever virucidal properties to a degree fully comparable with that of serum taken from individuals a few years after attacks of the disease. To see whether this supposedly non-specific power would protect against other virus diseases, Dr. Thomas M. Rivers and Dr. Leslie T. Webster, of The Rockefeller Institute for Medical Research, tested this serum for us by protection test in mice against the virus of lymphocytic choriomeningitis and that of St. Louis encephalitis, but found no protective properties against either.

Bacterial agglutinins and bactericidal substances have long been demonstrated in normal human and animal sera. More recently, trypanocidal substances in normal human serum have been reported by Culbertson (13) in cases in which the possibility of trypanosomal infection was definitely ruled out. Very little is known regarding the non-specific virucidal substances in normal human serum; that is, in the serum of persons never infected with the causative organism of the disease under consideration. It is true that protective substances against the viruses of herpes and poliomyelitis in normal human serum have been reported by numerous investigators, and a theory has been advanced by Jungeblut and Engle (14) and others that the occurrence of virucidal and other antibodies in normal human

serum is the result of normal physiological maturation of the human body. But as both these viruses are practically ubiquitous in their distribution, the possibility of a missed subclinical infection as the cause of immunity cannot be definitely ruled out. On the other hand, the possibility of contracting yellow fever in India can be safely ruled out. Also we know of no other infection which gives cross immunity to yellow fever. The possibility of cross immunity between yellow fever and dengue has been excluded. Specimens were received from Philippine Army scouts who had recently recovered from dengue, and some of the sera from Miami, Florida, were from persons who were convalescent from dengue in 1934. As shown in tables 1 and 2, neither group showed evidence of protection against yellow fever virus. Furthermore, the work of Snijders, Postmus, and Schüffner (15), and of Stefanopoulo and Callinicos (16) offers additional evidence that no cross immunity exists between yellow fever and dengue.

From the evidence accumulated in the course of this study of serum specimens from parts of the world where yellow fever presumably never occurred, we have reason to believe that yellow fever virucidal substances are present in the serum of some human beings who have not been exposed to yellow fever virus. If the protection test were made sufficiently sensitive, it would probably be possible frequently to demonstrate the presence of such virucidal substances in normal human serum in varying degrees of concentration, ranging from total absence to a level equal in rare instances to that of the active specific immunity following infection, as exemplified by the serum of "N" of India. Although of considerable academic interest, so sensitive a test would be of little value in epidemiological surveys. The test as performed now, using massive doses of virus, is highly specific. Had the present technique been developed when most of the sera shown in table 1 were tested, it seems probable that only one of the 876 specimens would have shown full protection. On the other hand, because the test is so severe, there is a probability that some specimens having small amounts of specific yellow fever immune substance due to past attacks of yellow fever may be missed. Absolute specificity could hardly be expected of any biological test involving the use of living animals.

COUNTRIES IN WHICH YELLOW FEVER IS ABSENT BUT OCCURRED IN THE MORE DISTANT PAST

From the historical records it is apparent that yellow fever was at one time present in epidemic form in parts of southern Europe and in the eastern and southern regions of the United States of America. It has also appeared several times as introduced cases in southeastern Canada. In none of these regions have there been outbreaks in recent years. A few cities were selected for investigation to see if the protection test would confirm the evidence of history that yellow fever is now completely absent. The results of the immunity survey are shown in table 2 and map II.

The historical data presented below with regard to yellow fever in Europe and North America are from Augustin (17). In Italy specimens were taken in Rome, Naples, and Leghorn. Yellow fever has never been observed in Rome or Naples, but there was a small outbreak at Torre Annunziata near Naples in 1883, the last recorded appearance of yellow fever on shore in Italy. In 1804 Leghorn suffered from a yellow fever epidemic in which 655 persons died, and the disease was brought to the port in 1821 and 1828 but did not spread. As would be expected, none of the specimens from Italy gave protection. The oldest blood donors in Leghorn and Naples were aged 74 and 64 years, respectively.

Spain and Portugal were repeatedly infected by ships coming from the Americas or West Africa, and many towns and cities were involved. The great epidemic in Barcelona in 1821 caused about 20,000 deaths. In Malaga in 1803 and 1804, yellow fever caused the death of 6884 and 11,486 persons, respectively. The last epidemic in that city, with 242 deaths, occurred in 1821. In 1890 there were 5 cases in persons who had visited a ship in the harbor, the last appearance of yellow fever in Spain. The age of the oldest of the Malaga blood donors was 63 years. In Valencia the only recorded cases were in sailors from Barcelona in 1870. The oldest donor was 66 years of age. Lisbon had many visitations, and its epidemic of 1857 caused 5652 deaths. The last introduction into Lisbon was in 1879, with the exception of one suspected case in 1880. The age of the oldest blood donor

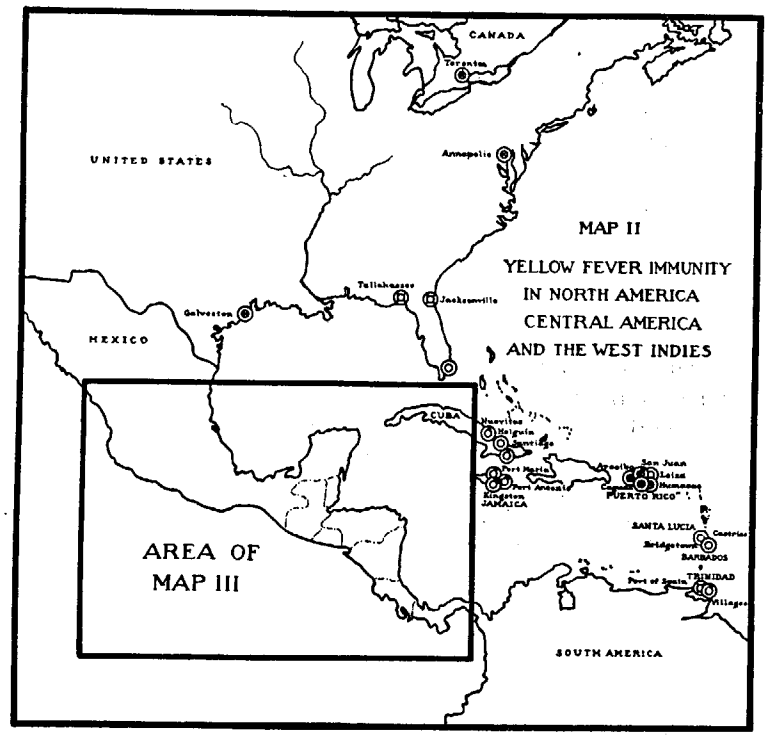
TABLE 2

Results of protection test survey in countries in which occasional epidemics or introduced cases were present in the more distant past

COUNTRY AND TOWN	AGE GROUPS OF DONORS												TOTAL FOR ALL AGE GROUPS			YEAR OF LAST EPIDEMIC	YEAR SPECIMENS COLLECTED
	5 to 9 years			10 to 14 years			15 to 19 years			Adults 20 years and over							
	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive		
Italy:																	
Rome							2			22			24				1935
Naples							2			13			15				1935
Leghorn							2			23			25			1828	1935
Spain:																	
Malaga										12			12			1890	1935
Valencia										30			30			1870	1935
Portugal, Lisbon										43			43			1880	1935
Canada, Toronto										109	1	0.92	109	1	0.92		1931
United States:																	
Annapolis, Md	8			15			25			38			63			1888	1932
Jacksonville, Fla	9			15			4			21			23			1899	1933
Miami, Fla	6			7						49			49			1867	1934
Tallahassee, Fla				1			5			69			13			1897	1933
Galveston, Texas													75				1933
Total	23			38			40			380	1	0.26	481	1	0.21		

in Lisbon was 60 years. All the specimens from Spain and Portugal were without protective power.

In the other parts of Europe, especially in Great Britain and France, yellow fever occurred only as isolated cases or very small



MAP II. DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN NORTH AMERICA, CENTRAL AMERICA, AND THE WEST INDIES

Symbols as in map I

outbreaks related to shipping and the disease did not spread freely among the people on land.

Canada has never had a true epidemic of yellow fever in the general population. There has never been a reported case in Toronto, where the serum specimens were collected from persons

from various parts of Canada. Some had traveled in countries known to be free from yellow fever. The disease is said to have been introduced into Quebec in 1805, 1812, and 1864; into Halifax in 1842, 1861, and 1878; and into Prince Edward Island in 1880. In Quebec there were said to have been 55 cases and 6 deaths in an army regiment in 1805. The age of the oldest blood donor was 79 years.

The one protective serum recorded in table 2 came from Canada and, like the two positive sera from India, it came from a person who in all probability had never been exposed. The first sera from Canada were tested early in 1932 when 10 per cent mouse-brain suspension was used regularly as the source of virus. The infected mouse brains used in testing some of these sera had been kept overnight in the frozen state in an icebox, contrary to our usual practice. Two specimens showed full protection, and seven gave inconclusive results in which only half of the test animals survived. These nine sera were retested with a fresh 10 per cent mouse-brain suspension, and all with the exception of one gave negative results, according to the present criteria which require that the result be classed as definitely negative if not more than two mice survived out of six or five. This one serum from "M. H." was tested twice more and continued to give partial protection each time. Five months later a second specimen was secured from the same person and was tested four times. The results were once negative, twice positive, and once inconclusive. In the final analysis this serum is classified as positive for yellow fever protection, although we feel that if a 20 per cent virus suspension had been used, the results might have been negative or inconclusive. The serum evidently had slight protective properties, probably non-specific.

In the United States of America only a few places were studied, and none of the moderate number of random samples of serum had protective power against yellow fever virus. The results are given in table 2. It had been shown previously by Sawyer (18) that protective sera could be obtained in the United States by seeking out aged persons who remembered having had yellow fever in the epidemics which formerly occurred in the United States, the last in 1905. In the present study the ages of the oldest blood donors in Annapolis, Miami, and Galveston were 58, 68, and 75 years, respectively. The donors from Jacksonville and Tallahassee were children. The limited evidence is consistent with the belief that yellow fever has not recently been present in the United States.

The specimens from Galveston, Texas, were supplied by Sharp and Hollar (19), who have discussed the significance of the protection test results. They reached the conclusion that the supposed cases of yellow fever in that city in 1897 were not of that disease but were more probably part of the prevailing epidemic of dengue. Serum from five persons who had had dengue and from one who supposedly had had yellow fever in that year were submitted in addition to the random samples and were found to be devoid of protective power against yellow fever virus.

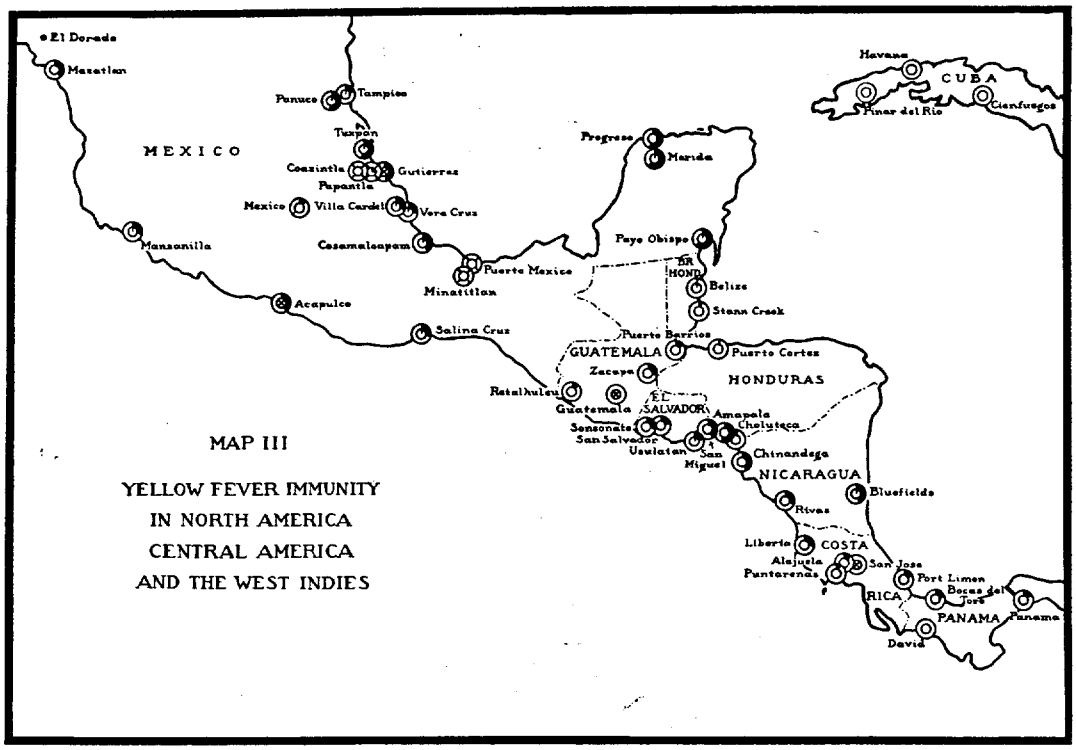
YELLOW FEVER IMMUNITY SURVEY IN THE WEST INDIES

The early American colonization records indicate that the islands of the Greater and the Lesser Antilles were hotbeds of yellow fever for at least three centuries. In some of them the disease seems to have been present continuously, while in others it was introduced from time to time. But after the mechanism of the transmission of yellow fever from man to man had become understood through the work of Walter Reed and his associates in Havana in 1900, and appropriate anti-mosquito measures had been gradually introduced, the disease disappeared from most of the islands within the first decade of the present century. In some of them, however, the disease persisted much longer, and cases were reported as late as 1921 in Martinique and Guadeloupe, but in recent years no new cases have been observed. As this history indicates, these islands are capable of playing a very important rôle in the spread of yellow fever to the American continent. They lie on important maritime trade routes and are regularly visited by ships from all over the world which are engaged in the Central and South American trade. They lie on the main air routes between North and South America. Moreover, some of the islands are in fairly close proximity to the South

TABLE 3

Results of protection test survey in countries in which occasional epidemics have occurred

ISLAND AND TOWN	AGE GROUPS OF DONORS												TOTAL FOR ALL AGE GROUPS			YEAR OF LAST EPIDEMIC	YEAR SPECIMENS COLLECTED
	5 to 9 years			10 to 14 years			15 to 19 years			Adults 20 years and over							
	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive		
Barbados, Bridgetown	21			21			26			5			73			1916	1934
Cuba:																1908	1936
Havana	47			134			22			21			224				
Santiago	9			15			3			22	2	9.0	49	2	4.1		
Holguin	16			8			5			19			48				
Nuevitas	5			19			6			20	2	10.0	50	2	4.0		
Cienfuegos	11			13			4			22			50				
Pinar del Rio	2			43			17			21			83				
Jamaica:																1905	1934
Kingston	1			20			6			25	9	36.0	52	9	17.3		
Port Antonio				27			2			19	3	15.8	48	3	6.3		
Port Maria	1			26			2			27	3	11.1	56	3	5.4		
Puerto Rico:																1900	1931, 1932
San Juan							5			59	9	15.3	64	9	14.1		
Caguas										39			39				
Arecibo							1			11			12				
Humacao				1			6			20			27				
Loiza	4			9			23			19			55				
St. Lucia, Castries	29			17			12			5			63				1934
Trinidad:																1914	1934
Port of Spain	22			44			8						74				
San Fernando	19			13			12			2	2	100.0	46	2	4.3		
La Brea	18			19			4						41				
Tunapuna	6			17									23				
Total	211			446			164			356	30	8.42	1,177	30	2.55		



MAP III. DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN PARTS OF MEXICO, CENTRAL AMERICA, AND CUBA

Symbols as in map I

American mainland, in the interior of which yellow fever is endemic at the present time. For these reasons, it was considered important that an immunity survey be undertaken to determine whether or not yellow fever is still endemic in certain representative islands. Accordingly, serum specimens were secured for yellow fever protection tests from persons of various age groups living in Barbados, Cuba, Jamaica, Puerto Rico, St. Lucia, and Trinidad.

The results of this survey are summarized in table 3 and maps II and III. As seen from the tabulated summary, a total of 1177 specimens were examined. All of 821 sera from persons under 20 years of age gave negative results. Of 356 sera from adults of 20 years of age or over, there were 30 specimens, or 8.42 per cent, which showed definite protection against yellow fever virus. The majority of the protecting sera were from persons over 40 years of age. The youngest adult showing immunity to yellow fever was a Cuban, 28 years old. According to his age, this donor was born in 1908, which is the year when the last case of yellow fever was reported from Cuba, and he may have had an immunizing attack in his early infancy. Recio (20) reported testing a total of 41 sera from donors ranging from 7 to 70 years of age in Cuba, and found 12 positive for yellow fever immunity, the youngest immune donor being 36 years old. The protection test results and the disease records together seem to indicate that the disease has not been recently present in the West Indies.

YELLOW FEVER IMMUNITY SURVEY IN MEXICO AND CENTRAL AMERICA

It is generally believed that yellow fever was long endemic in Mexico and in Central America, and Boyce (21) is inclined to think that it was present more or less continuously from the sixteenth century to the time of the publication of his treatise (1911). Although explosive epidemic outbreaks were relatively infrequent, there were sporadic cases occurring practically all the time, which suggested endemicity. When there was a large influx or accumulation of non-immune human subjects, the disease tended to assume epidemic form. The tragedy which overtook the early builders of the Panama Canal is familiar to everyone. The last widespread epidemics occurred in 1919, 1920, and 1921, when numerous cases of yellow fever appeared over a wide area in Mexico and Central America. After the principal epidemics had died down, an outbreak occurred in Salvador in 1924 and there were a few cases in Guatemala and British Honduras in that year. Since then occasional cases of fever with jaundice have been encountered, but none has been definitely diagnosed as yellow fever. In fact, we had opportunity to test sera from some of these cases and found no immunity to yellow fever.

In the course of the survey of Mexico and Central America, a total of 2271 serum specimens were examined. They were collected in 44 different towns or communities, and ages from 5 to 70 years were represented among the donors. The results of the protection tests with these sera as well as the number of specimens tested from each locality are summarized in tables 4 and 5.

The survey of Mexico was begun early in 1932, and most of the results have already been analyzed by Mejia (22). Since Dr. Mejia's report was written, however, additional specimens have been tested, and the results of the entire survey are summarized in table 4. This tabulated summary and map 3 require little explanation, but there are a few points which need further elucidation. It will be noticed that three sera among the 321 obtained from children under ten years of age showed protection against yellow fever virus. These specimens came from Merida, Tampico, and Manzonillo in 1932, and the ages of the donors at that time were given as 8, 8, and 7 years respectively. If the ages of these donors were given correctly, two of the children must have been born in 1924 and one in 1925, i.e., two and three years after the last recognized case of yellow fever in Mexico. It is difficult to explain the yellow fever immunity in these children supposedly born several years after the disease had apparently disappeared from their country. Several possibilities suggest themselves, however. These sera were tested early in 1932 when 10 per cent brain-virus suspension was routinely used. As pointed out above, the test as performed then was slightly oversensitive and occasionally produced false positives. It would

TABLE 4

Results of protection test survey in Mexico

CITY AND STATE	AGE GROUPS OF DONORS										TOTAL FOR ALL AGE GROUPS			YEAR OF LAST EPIDEMIC	YEAR SPECIMENS COLLECTED
	5 to 9 years			10 to 14 years			15 to 19 years			Adults 20 years and over					
	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive		
Mexico City, D. F.	27	1	3.7	25			3			22	3	13.7	50	3	6.0	1921	1934
Manzanillo, Colima				13			6	2	33.3	18	5	27.8	64	8	12.5	1921	1932, 1934
Salina Cruz, Oaxaca				24	1	4.2	4	1	25.0	16	5	31.2	44	7	15.9	1920	1934
Acapulco, Guerrero	12						10	2	20.0	15	8	42.1	19	8	42.1	1921	1932
Mazatlan, Sinaloa				13						5	1	20.0	5	1	20.0	1921	1934
El Dorado, Sinaloa	10									15	10	66.6	40	11	27.5	1921	1932
Payo Obispo, Campeche				14	1	7.1	1			36	8	22.2	94	9	9.6	1921	1934
Tampico, Tamaulipas	24	1	4.2	32			2			40	26	65.0	116	29	25.0	1920	1932, 1934
Merida, Yucatan	54	1	1.8	17			5	2	40.0	24	14	58.4	50	15	30.0	1920	1934
Progreso, Yucatan	16			8			2	1	50.0	21	7	33.3	100	10	10.0	1921	1932, 1934
Vera Cruz, Veracruz	48			26	3	11.5	5						31			1914	1934
Puerto Mexico, Veracruz	3			28						16	6	37.5	78	11	14.1	1921	1934
Villa Cardel, Veracruz	24			32	3	9.4	6	2	33.3	13	2	15.4	77	15	19.5	1921	1934
Cosamaloapam, Veracruz	25			31	6	19.3	8	7	87.5	25	14	56.0	75	18	24.0	1921	1934, 1936
Panuco, Veracruz	23			27	4	14.8				7	2	28.6	54	14	25.9	1921	1934, 1936
Papantla, Veracruz	6			39	12	30.8	2			21	13	62.0	77	16	20.8	1921	1934, 1936
Tuxpan, Veracruz	29			25	3	12.0	2						24			1914	1934
Minatitlan, Veracruz	6			18			3			10	6	60.0	13	6	46.2	1921	1934
Gutierrez, Veracruz				14									28			1921	1936
Coazintla, Veracruz	14																
Total	321	3	0.90	386	33	8.55	59	17	28.81	323	138	42.72	1,089	191	17.53		

seem remotely possible that the protective substances in all three of these sera were of the non-specific variety, and not acquired as a result of infection with yellow fever virus. Unfortunately, this possibility could not be further explored as we have not been able to secure second specimens from any of these children. Dr. Mejia has suggested that the ages may have been incorrectly given and that these three children were probably older than stated. There remains another possibility, that the yellow fever infection in a mild unrecognized form lingered in the communities for several years after the last epidemics had died down. There were no protective specimens, however, among those obtained from children born after the year 1925, and as there have been no definitely diagnosed cases of yellow fever reported since 1922, it would seem reasonably safe to conclude that yellow fever is not endemic in Mexico at the present time and has probably been completely absent from there during the last ten years.

As seen from the tabulated summary, the proportion of immunes in Mexico increases progressively with the age of the donors. In the age group of 10 to 14 years, 8.55 per cent gave full protection among 386 specimens tested; of 59 sera from donors 15 to 19 years of age 28.81 per cent were positive, and for the adults of 20 years and over the percentage became 42.72. As a result of repeated exposure to epidemics, the percentage of immunes in some localities was very high, as for example in Merida, where 26 of 40 adults, or 65 per cent, and in Payo Obispo where 10 of 15 adults, or 66.6 per cent, proved to be immune.

The results of the survey in Central America, shown in table 5, correspond in general with those obtained in Mexico. The distribution of immunes among the various age groups is similar, although the percentage is somewhat lower than in Mexico. It will be noticed in table 5 that of a total of 135 sera from children under 10 years of age there was one protective specimen from Salvador, bringing the percentage of immunes for this age group to about the level found in Mexico. This positive serum came from a child in Usulatan, Salvador, in 1934, when 20 per cent virus was being used in all tests. At that time the child was said to be 9 years old, which would mean that he was born in 1925.

TABLE 5

Results of protection test survey in Central America

COUNTRY AND TOWN	AGE GROUPS OF DONORS												TOTAL FOR ALL AGE GROUPS			YEAR OF LAST EPIDEMIC	YEAR SPECIMENS COLLECTED
	5 to 9 years			10 to 14 years			15 to 19 years			Adults 20 years and over							
	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive		
1. El Salvador:																	
Usulatan	3	1	33.3	19	1	5.3	6	1	16.7	15	4	26.7	43	7	16.3	1921	1934
Sonsonate	3			8			12	1	8.3	20	9	45.0	43	10	23.2	1919	1934
San Miguel	6			17			16	3	18.8	17	11	58.8	56	14	25.0	1920	1934
San Salvador	13			17			6	1	16.7	17	4	23.6	53	5	9.4	1924	1934
2. Guatemala:																	
Retalhuleu	11			19			10			26	5	19.2	66	5	7.6	1918	1935
Puerto Barrios	6			13			4	1	25.0	8	1	12.5	31	2	6.5	1910	1935
Zacapa	9			8			13	2	15.4	37	12	32.4	67	14	20.9	1924	1935
Guatemala City				1			3			16			20			?	1935
3. British Honduras:																	
Stann Creek	1			14			20	1	5.0	14	1	7.1	49	2	4.1	1921	1936
Belize	5			14	1	7.1	5			21	8	38.1	45	9	20.0	1924	1936
4. Honduras:																	
Puerto Cortez	5			10			11			21	1	4.8	47	1	2.1	1905	1936
Choluteca	6			11			12			13	1	7.7	42	1	2.4	1919	1936
Amapala	10			13			6	1	16.7	23	15	65.2	52	16	30.8	1921	1936
5. Nicaragua:																	
Bluefields	12			29	4	13.8	14	5	35.7	21	11	52.4	76	20	26.3	?	1934
Rivas	4			15			11	1	9.1	20	9	45.0	50	10	20.0	1919	1934
Chinandega	6			26			4			20	11	55.0	56	11	19.6	1919	1934
6. Costa Rica:																	
San Jose	2			3			10			2			17			1910	1932
Alajuela	4			6			13			23	5	21.7	46	5	10.9	1899	1934
Liberia	5			15			5			20	7	35.0	45	7	15.5	1901	1934
Puntarenas	2			11			10			15	2	13.3	38	2	5.3	1902	1934
Port Limon	4			17			8			15	4	26.7	44	4	9.1	1910	1934
7. Panama:																	
David	3			15			15			12			45			?	1934
Bocas del Toro	9			8			13			26	8	30.8	56	8	14.3	1905	1934
Canal City and Panama City	6			8			17			64	13	20.3	95	13	13.7	1905	1934
Total	135	1	0.74	317	6	1.90	244	17	7.00	486	142	29.22	1,182	166	14.04		

According to the records available, yellow fever was reported from San Salvador, the capital of the republic, as late as 1924, but no cases are known to have occurred in Usulatan, the town in which the child lived, since 1921. Here again, as with the three similar specimens from Mexico, we can offer no definite explanation for this finding. We believe, however, that the factors, whatever their nature, which enabled us to demonstrate yellow fever virucidal substances in the blood of the three children of the same age group in Mexico, are also responsible for the positive finding in this instance, and we are inclined to the conclusion that yellow fever persisted for a time after the last recorded cases. As with the Mexican cases, we were unable to secure another specimen of serum from this child and the test therefore could not be repeated.

In studying the results in table 5, it will be noticed that of the seven countries included in this survey, Costa Rica and Panama stand out in sharp contrast to the others. In Costa Rica yellow fever has not been reported since 1910 in any of the five towns where sera were obtained for the immunity survey. For some unknown reason these towns apparently escaped infection during the epidemics of 1919, 1920, and 1921, which were widespread over Central America. This is fully corroborated by the protection test results. It will be noticed that there were no immunes among the 115 persons under 20 years of age examined from Costa Rica. In Panama the last case of yellow fever occurred in 1905, and none of the 21 donors of protective serum was born after that date. The Panamanian territory south of the Canal Zone is being studied at the present time.

On the basis of the data presented above, we are inclined to the conclusion that yellow fever is not endemic at present in the American continent north of the Panama Canal. In fact, our results indicate that the disease was widespread in Mexico and Central America in the past but probably disappeared entirely within recent years. It is true that the sera from very young children tested in the course of this survey have been relatively few considering the wide territory covered, but nevertheless the findings seem significant because in them are represented the

cities and towns which are of greatest interest from an epidemiological standpoint. Most of these cities are either important centers of communication or places which were known as notorious hotbeds of yellow fever until relatively recent years. Whether the disease may be present in some limited rural areas in the form of the so-called "jungle yellow fever" recently reported from Brazil and Colombia (Soper (7, 23, 24)) is impossible to determine definitely from the results of our survey. But the absence of immunity in children born within the last ten years as well as the absence of recognized cases from both urban and rural communities during this period would seem to render the existence of this type of yellow fever in Mexico and Central America highly improbable.

SUMMARY

1. Evidence is presented to show that the yellow fever protection test in mice, as used in epidemiological surveys, is highly specific.

2. Of a total of 876 human sera from Asia and Australia, where yellow fever has presumably never been present, only 2 specimens, or 0.23 per cent, showed protection against yellow fever virus. Among 481 sera from Italy, Spain, Portugal, Canada, and the United States, where yellow fever was formerly present but is now absent, only one was protective.

3. A total of 1177 sera were tested from the following islands of the West Indies: Barbados, Cuba, Jamaica, Puerto Rico, St. Lucia, and Trinidad. Among them were 821 from persons under 20 years of age, none of whom gave blood-serum which was protective against yellow fever virus. Of 356 sera from adults over 20 years old, 30, or 8.42 per cent, showed immunity to yellow fever.

4. A total of 1089 sera were tested from Mexico. There was a large percentage of protective sera from donors of all age-groups except very young children. All sera from children born after the year 1925 gave negative results, suggesting that yellow fever has probably disappeared from Mexico within recent years.

5. A total of 1182 specimens were tested from the seven Cen-

tral American countries. The results for El Salvador, Guatemala, British Honduras, Honduras, and Nicaragua were similar to those for Mexico. The sera from persons under 20 years of age in Panama and Costa Rica gave no protection against yellow fever virus, while the percentage of immunes in the older persons considered as one group was about the same as in the other Central American countries.

We wish to express our appreciation for the whole-hearted cooperation of the medical authorities and other persons of the various countries included in this survey who were instrumental in collecting and sending the sera for protection test. Needless to say, without their coöperation these studies would not have been possible. We received specimens from the places mentioned through the courtesy of the following: Dr. Jean Macnamara and Dr. Margaret Ashton from Melbourne, Australia; Dr. Nichols and Dr. R. Briercliffe from Colombo, Ceylon; Dr. J. B. Grant, Dr. H. L. Amoss, and Dr. I. C. Fang from Peiping, China; Dr. C. Bonne, Dr. W. M. Bonne, and Dr. Pet from Java; Dr. K. S. Shah, Lieut. Col. R. Knowles, Lieut. Col. S. S. Sokhey, Dr. C. G. Pandit, Lieut. Col. H. H. King, Major W. J. Webster, and Dr. Vankatraman from India; Dr. Peter H. Martin and Dr. A. W. Kingsbury from the Federated Malay States; Major G. C. Dunham of the U. S. Army Medical Corps, from the Philippines; Dr. H. A. Yenikomshian from Syria; Dr. Lewis W. Hackett from Italy; Dr. Rolla B. Hill from Spain and Portugal; Dr. J. G. FitzGerald and Dr. D. T. Fraser from Toronto, Canada; Dr. Henry Hanson, Dr. G. N. MacDonell, and Dr. Mark F. Boyd from Florida; Dr. J. H. Janney from Maryland; Prof. W. B. Sharp and Dr. E. D. Hollar from Texas; Dr. A. M. Walcott from the islands of Barbados, St. Lucia, and Trinidad; Dr. H. P. Carr from Cuba and Mexico; Dr. B. E. Washburn and Dr. T. B. Turner from Jamaica; Dr. G. C. Payne, Dr. O. Costa Mandry, and Dr. Silva from Puerto Rico; Dr. C. A. Bailey from Mexico; Dr. D. M. Molloy from Central America; Col. J. F. Siler and Dr. L. B. Bates from Panama.

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